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Antioxidant and prooxidant activities of phenolic acids commonly existed in vegetables and their relationship with structures

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Abstract

Phenolic acids existed in many agricultural products especially in vegetables and considered as important natural antioxidants. Eleven phenolic acids existed commonly in vegetables were chosen to assess the antioxidant activities using DPPH·, ·ABTS$^+$, ·O_2^-, and ·OH scavenging assays, as well as ferric reducing power and β-carotene bleaching assays. The results showed that caffeic and protocatechuic acid with simple dihydroxylation structures have the best antioxidant activities, while *p*-Hydroxybenzoic and *p*-Coumaric acid with mono hydroxylation structures have the lowest antioxidant activities in most assays. The antioxidant activity of caffeic acid was higher than protocatechuic acid and ferulic acid higher than isoferulic acid may be due to the substituents and the position of substituents on the benzene ring. Antioxidant activities of caftaric, chicoric and chlorogenic acid and its derivates with complex dihydroxylation structures were high relatively among phenolic acids. The different concentration ranges of phenolic acids were obtained based on the quantitative relationship between antioxidant or prooxidant activities and sample concentrations. The prooxidant effect of phenolic acids was rarely observed in the present study. The results were conducive to further research on potential biological activities of vegetables.

Keywords: antioxidant activity; phenolic acid; vegetable; structure; prooxidant activity.

Practical Application: The article reveals that caffeic and protocatechuic acid with simple dihydroxylation structures have the best antioxidant activities. Antioxidant activities of caftaric, chicoric and chlorogenic acid and its derivates with complex dihydroxylation structures were high relatively among phenolic acids. The results will provide a meaningful reference for further research on phenolic acids, and for design of fortified food or nutraceuticals with high antioxidant activities.

1 Introduction

Vegetables are an important part of healthy diet and have been proved to have tight relationship with human nutrition and health for containing of various phytochemical constituents (Lafay & Gil-Izquierdo, 2008; Sevgi et al., 2015; Sarker & Oba, 2020; Negrão et al., 2021). Phenolic acids including hydroxybenzoic and hydroxycinnamic acids and their derivatives are important secondary metabolites in vegetables (Robbins, 2003), especially hydroxycinnamic acids, occurring in many vegetables make significant contributions to the polyphenol intake (Clifford, 2004; Maciej et al., 2020).

The most common hydroxycinnamic acids are caffeic, *p*-coumaric and ferulic acids, chlorogenic acid and its derivatives were also found frequently in vegetables (Khanam et al., 2012; Mattila & Hellström, 2007). Chlorogenic acid is combined from caffeic and quinic acids and its derivatives differ in the patterns of the hydroxylations of their aromatic rings (Shahidi & Naczk, 2003). Ferulic, isoferulic and *p*-coumaric acid were higher than other phenolic acids found in leafy vegetables (Zhang et al., 2019). Caftaric and chicoric acids are commonly existed in Lamiaceae Vegetables according to Prommajak et al. (2016) Hydroxybenzoic acids in vegetables are generally low (Shahidi & Naczk, 2003) with p-hydroxybenzoic and protocatechuic are the most common forms found in vegetables (Maurya & Devasagayam, 2010). Phenolic acids existed commonly in vegetables were listed in Table 1.

According to previous research, phenolic acids have attracted considerable interest in the past few years due to their powerful antioxidant activities (Priyadarsini et al., 2002; Reber et al., 2011; Palafox-Carlos et al., 2012; Guedes et al., 2022; Plesoianu et al., 2021). The ability of phenolic acids to act as antioxidants under in vitro system is dependent on several factors such as concentration, structure and the test system used.

Many antioxidants can be made to exert prooxidant effects in vitro under certain conditions (Maurya & Devasagayam, 2010), the relationship between antioxidant and prooxidant effect of phenolic acids intimately depends on their concentration (Parker et al., 2010; Sevgi et al., 2015; Yen et al., 2002). Caffeic and chlorogenic acids had been proved to accelerate LDL oxidation in the propagation phase at lower concentrations and inhibit LDL oxidation at higher concentrations (Yamanaka et al., 1997).

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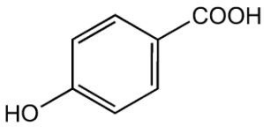
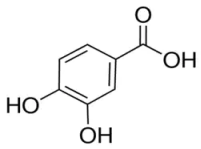
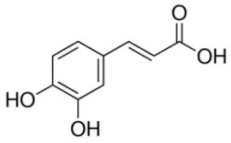
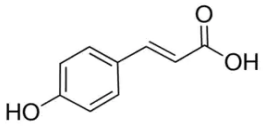
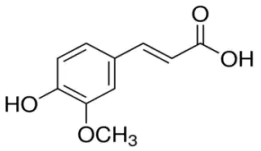
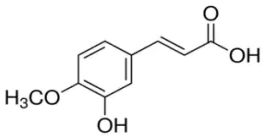
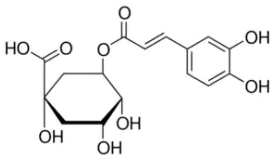
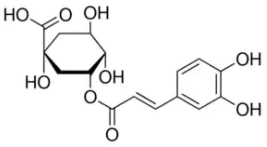
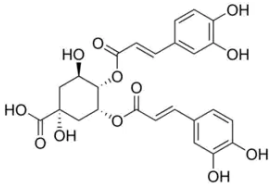
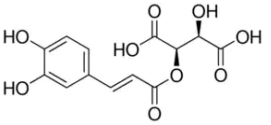
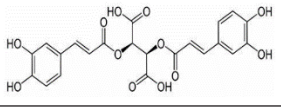
Table 1. Phenolic acids commonly existed in vegetables.

Phenolic acids	Molecular formula	Molecular Structure
p-Hydroxybenzoic acid (*p*-HBA, 4-Hydroxybenzoic acid)	$C_7H_6O_3$	
Protocatechuic acid (PCA, 3,4-Dihydroxybenzoic acid)	$C_7H_6O_4$	
Caffeic acid (CA, 3,4-dihydroxycinnamic acid)	$C_9H_8O_4$	
p-Coumaric acid (*p*-CA, 4-Hydroxycinnamic acid)	$C_9H_8O_3$	
Ferulic acid (FA, 4-Hydroxy-3-methoxycinnamic acid)	$C_{10}H_{10}O_4$	
Isoferulic acid (IFA, 3-Hydroxy-4-methoxycinnamic acid)	$C_{10}H_{10}O_4$	
Chlorogenic acid (3-CQA, 3-*O*-Caffeoylquinic acid)	$C_{16}H_{18}O_9$	
Neochlorogenic acid (5-CQA, 5-O-Caffeoylquinic acid)	$C_{16}H_{18}O_9$	
Isochlorogenic acid A (ICQA, 3,5-dicaffeoylquinic acid)	$C_{25}H_{24}O_{12}$	
Caftaric acid (CTA, Caffeoyl tartaric acid)	$C_{13}H_{12}O_9$	
Chicoric acid (DCTA, Dicaffeoyl tartaric acid)	$C_{22}H_{18}O_{12}$	

Nevertheless, Maurya & Devasagayam reported that caffeic and ferulic acids behave as pro-oxidants in fenton reaction when at higher concentrations (2010). It was questioned whether the phenolic acids served as potent antioxidants can also display pro-oxidant activity at certain concentrations or in certain determination methods.

The relationship between the chemical structure and the anti-oxidant/prooxidant activities has also been the focus of important studies. The chemical structure of phenolic acids gives them the ability to act as free radical scavengers. The type of compound, the number of hydroxyl groups and the degree of methoxylation are some of the parameters that determine the antioxidant activity (Yordi et al., 2012). Chen et al. (2020) investigated the antioxidant activities of typical phenolic acids and discovered that based on the same substituents on the benzene ring, phenolic acids with -CH_2COOH and -CH = CHCOOH can enhance antioxidant activities, compared with -COOH. Furthermore, methoxyl (-OCH_3) and phenolic hydroxyl (-OH) groups can also promote antioxidant activities of phenolic acids. Yang et al. (2021) found methoxyl on the benzene rings had positive effects on the antioxidant activity of disinapic acids and diferulic acids by experiments and theoretical calculation.

Additionally, antioxidant activities of the results from different studies on antioxidant abilities of phenolic acids were hard to compare and analysis because of different determination methods or different modifications and reference materials in same method. Some discrepancies were found in the characterization of antioxidant activities for individual phenolic acids in former researches (Chen & Ho, 1997; Li et al., 2011; Sevgi et al., 2015; Maciej et al., 2020). The consistency of the determination method and experimental conditions is particularly important for the comparison of antioxidant activities among the phenolic acids. Beyond that, antioxidant activities of caftaric and chicoric acids were rarely studied in the literature though they commonly existed in many *Lamiaceae* vegetables (Prommajak et al., 2016).

Thus, in the present study, the antioxidant activities of 11 phenolic acids commonly found in vegetables were measured by six unified methods including DPPH·, ·$ABTS^+$, ·O_2^- and ·OH scavenging assay, ferric ion reducing antioxidant power (FRAP) assay and β-carotene bleaching assay. Relationship between the antioxidant activities and the structure and concentration of phenolic acids was also studied. This study would help to provide relatively unified and comprehensive information for the antioxidant activities of phenolic acids and conduct further research on potential biological activities of vegetables.

2 Materials and methods

2.1 Reagents

2, 2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 1, 1-diphenyl-2-picryl-hydrazyl (DPPH), nitroblue tetrazolium (NBT), reduced ß-nicotinamide adenine dinucleotide (NADH), phenazine methosulfate (PMS), ethylenediamine tetra-acetic acid disodium salt (Na_2-EDTA), thiobarbituric acid (TBA), trichloroacetic acid (TCA), 2, 4, 6-tris(2-pyridyl)-s-triazine (TPTZ), β-carotene, linoleic acid, L-ascorbic acid, Tween-20, butylated hydroxytoluene (BHT), D-2-deoxyribose, 2, 2'-azobis(2-methylpropionamidine)-dihydrochloride (AAPH) and phenolic acids involved were all purchased from Sigma-Aldrich Chemical Co (Shanghai, China). All chemicals and solvents were of analytical grade.

2.2 Experimental methods

DPPH radical scavenging assay

The determination of DPPH radical scavenging activity was based on the method described by Liang et al. (2019) with some modifications. Briefly, 1 mL phenolic acids or BHT solutions at various concentrations (0.5-10 μg/mL) in methanol were added to 2 mL DPPH solution (100 μM in methanol), respectively, then the mixture was shaken vigorously and kept at room temperature for 30 min in the dark. The absorbance was measured at 517 nm using Alpha-1506 UV–Visible spectrophotometer (Purkinje General, Beijing, China). The DPPH radical scavenging activity was calculated using the following Equation 1:

$$DPPH\cdot \text{ scavenging rate } (\%) = \left[\left(A_C - A_S \right) / A_C \right] \times 100 \quad (1)$$

where A_C is the absorbance of the negative control at 517 nm and A_S is the absorbance of the presence of phenolic acids or BHT.

ABTS radical scavenging assay

The ABTS radical scavenging assay was carried out according to Thaipong et al. (2006) with modifications using multimode reader instead of the spectrophotometer. The ABTS radical cation was generated by the reaction of 7.4 mmol/L ABTS diammonium salt and 2.6 mmol/L potassium persulfate. The mixture was reacted for 12 h at room temperature in the dark. The ABTS solution was diluted with methanol (1: 50, V/V) to get the working concentration before using. 40 μL phenolic acids or BHT solutions and 160 μL ABTS solution were added to 96-well microplate and shaken slightly for 6 min using BE-9010 model thermo-shaker (Qilinbeier instrument manufactural Co., Ltd., Haimen, Jiangsu Province, China). The absorbance was measured at 734 nm by multimode reader (Multiskan FC, Thermo Scientific, USA). The ABTS radical scavenging activity was calculated as follows (Equation 2):

$$\cdot ABTS^+ \text{ scavenging rate } (\%) = \left[\left(A_C - A_S \right) / A_C \right] \times 100 \quad (2)$$

where A_C is the absorbance of the negative control at 734 nm and A_S is the absorbance of the presence of phenolic acids or BHT.

Superoxide anion scavenging assay

Superoxide anion scavenging activity was determined as described by Gülçin (2006) with some modifications. 30 μL phenolic acids or BHT solutions were added with 90 μL of 166 μmol/L NADH and 90 μL of 50 μmol/L NBT into 96-well microplate and shaken slightly. The 90 μL of 10 μmol/L PMS was

added to the mixture and kept at room temperature for 5 min. The absorbance was measured at 560 nm by multimode reader. Decreased absorbance of the reaction mixture indicated the increasing of superoxide anion scavenging activity. The superoxide anion scavenging activity was calculated as follows (Equation 3):

$$\cdot O_2^- \ scavenging\ rate(\%) = \left[\left(A_C - A_S \right) / A_C \right] \times 100 \quad (3)$$

where A_C is the absorbance of the negative control and A_S is the absorbance of the presence of phenolic acids or BHT.

Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity was determined using deoxyribose method described by Li et al. (2011) with some modifications. 1 mL phenolic acids or BHT solutions at various concentrations in methanol was added into mini tubes and blew by nitrogen to remove the solvent. 1.6 mL reaction reagent including 2.8 mmol/L deoxyribose, 0.025 mmol/L $FeCl_3$, 0.08mmol/L Na_2EDTA, 2.8 mmol/L H_2O_2 and 0.1 mmol/L ascorbic acid was added into the tube and incubated at 50 °C in water bath for 30 min. After incubation, 0.5 mL 2-thiobarbituric acid (TBA, 1%) and 0.5 mL trichloroacetic acid (TCA, 5%) were added into the mixture and kept in boiling water bath for 20 min. Then 200 μL reaction solution was added into the 96-well plate after cooling to room temperature. The absorbance was measured at 532 nm using multimode reader. The hydroxyl radical scavenging activity was expressed as Equation 4:

$$\cdot OH \ \ inhibition\ rate(\%) = \left[\left(A_C - A_S \right) / A_C \right] \times 100 \quad (4)$$

where A_C is the absorbance of the negative control and A_S is the absorbance of the presence of phenolic acids or BHT.

FRAP assay

The FRAP assay was used to measure the reducing activity of phenolic acids. As described by Santiago-Saenz et al. (2020), the FRAP reagent was obtained as follows: 25 mL of 0.3 mol/L sodium acetate buffer (pH 3.6), 2.5 mL of 10 mmol/L TPTZ in 40 mmol/L HCl, and 2.5 mL 10 mmol/L ferric chloride were mixed together and warmed to 37 °C in water bath. 10 μL phenolic acids or BHT solutions at various concentrations and 190 μL FRAP reagent were added to 96-well plate and kept at room temperature for 30 min in the dark. The absorbance was determined by multimode reader at 595 nm. The increased absorbance indicated increased reducing power.

β-carotene bleaching assay

β-carotene bleaching assay was performed as the method described by Fukumoto & Mazza (2000) with slight modifications. Stock solution of β-carotene-linoleic acid was prepared as follows: 10 mg β-carotene was dissolved in 10 mL chloroform, 1 mL solution was added into a tube with 0.1 mL linoleic acid and 1 g Tween 20 and blew by nitrogen to remove chloroform, then 100 mL distilled water was added into the tube and shaken vigorously. 200 μL stock solution was injected to 96-well plate with 20 μL phenolic acids or BHT solutions at various concentrations, then 20 μL of 0.3 mmol/L AAPH was added into it to start the reaction at 25 °C. The absorbance of reaction mixture was measured at 0 min and 90 min of reaction time at 470 nm on a multimode reader. The results were expressed as follows (Equation 5):

$$\beta - carotene\ bleaching\ inhibition\ rate\ (\%) = \left[1 - \left(A_0 - A_1 \right) / \left(C_0 - C_1 \right) \right] \times 100 \quad (5)$$

where A_0 and A_1 are the absorbance of phenolic acids or BHT solutions at the reaction time of 0 min and 90 min, respectively. C_0 and C_1 are the absorbance of the control at at the reaction time of 0 min and 90 min, respectively.

2.3 Statistical analysis

The data was expressed as mean ± standard deviation (SD) of three measurements. All figures in this paper were made by Origin 9.0 professional software.

3 Results

3.1 DPPH· and ·ABTS⁺ scavenging activity

DPPH· and ·ABTS⁺ are two synthetic free radicals, which have been used widely to determine the free radical scavenging activity of various antioxidants. The solutions of DPPH· and ·ABTS⁺ showed characteristic absorptions at 517 nm and 734 nm, respectively. The scavenging activities of DPPH· and ·ABTS⁺ were determined by measuring the decrease in the absorbance caused by the addition of antioxidants.

The EC_{50} and EC_{90} values were calculated from the linear curves of DPPH· and ·ABTS⁺ scavenging assays. As can be seen in Table 2, CA and PCA with dihydroxylation structure had remarkable abilities to scavenge DPPH· and ·ABTS⁺. The derivatives of caffeic acid, quinic acid and tartaric acid such as ICQA, 3-CQA, 5-CQA and DCTA also showed high scavenging activities in two assays. Moreover, the phenolic acids mentioned above were exhibited higher activities than BHT in DPPH· scavenging assays. IFA, *p*-CA and the phenolic acids mentioned above were showed higher activities than BHT in ·ABTS⁺ scavenging assays. *p*-HBA with mono hydroxylation structure showed the lowest activity among the phenolic acids in these assays. Maciej et al. (2020) also reported that mono hydroxylated compounds exhibited the lowest efficiency as antioxidants, while compounds with two or more hydroxyl groups in ortho or para position to each other illustrated the highest antioxidant properties. The chlorogenic acid isomers including ICQA, 3-CQA and 5-CQA showed higher activities of DPPH· scavenging than that of ·ABTS⁺ scavenging with the same order from high to low as ICQA>3-CQA>5-CQA. It was in accordance with the former research by Xu et al. (2012). The number of dihydroxylation structure maybe related to the antioxidant activities, ICQA has two dihydroxylation structure while 3-CQA and 5-CQA has one dihydroxylation structure in it. FA had been studied a lot for its antioxidant activity by many

Table 2. DPPH and ABTS radicals scavenging activity of phenolic acids (μg/mL).

Phenolic acids	DPPH		ABTS	
	EC_{50}	EC_{90}	EC_{50}	EC_{90}
p-HBA	176.9 ± 8.85 [a]	333.7 ± 16.69 [a]	39.87 ± 1.99 [a]	79.23 ± 3.96 [a]
PCA	4.06 ± 0.10 [e]	8.87 ± 0.44 [e]	4.25 ± 0.21 [f]	9.21 ± 0.46 [g]
CA	2.03 ± 0.08 [e]	4.76 ± 0.19 [e]	3.65 ± 0.15 [fg]	8.78 ± 0.25 [g]
p-CA	69.25 ± 2.08 [b]	134.5 ± 4.04 [b]	4.02 ± 0.12 [fg]	15.31 ± 0.46 [f]
FA	5.64 ± 0.39 [e]	12.36 ± 0.87 [e]	3.08 ± 0.22 [g]	8.57 ± 0.60 [g]
IFA	23.74 ± 1.42 [c]	46.07 ± 2.76 [c]	8.67 ± 0.52 [de]	18.26 ± 1.10 [de]
3-CQA	4.8 ± 0.29 [e]	10.79 ± 0.65 [e]	8.68 ± 0.52 [de]	17.83 ± 1.07 [de]
5-CQA	5.39 ± 0.16 [e]	11.53 ± 0.35 [e]	9.26 ± 0.28 [d]	19.01 ± 0.57 [d]
ICQA	4.57 ± 0.18 [e]	10.08 ± 0.40 [e]	7.97 ± 0.32 [e]	17.12 ± 0.68 [def]
CTA	25.5 ± 1.28 [c]	47.15 ± 2.36 [c]	12.53 ± 1.03 [b]	25.72 ± 2.29 [b]
DCTA	4.44 ± 0.18 [e]	10.18 ± 0.41 [e]	7.92 ± 0.32 [e]	16.33 ± 0.65 [ef]
BHT	17.15 ± 0.69 [d]	31.24 ± 1.25 [d]	11.17 ± 0.45 [c]	23.43 ± 0.94 [c]

EC_{50} and EC_{90} represent efficient antioxidant concentration (μg/mL) for scavenging 50% and 90% radicals. Each value is expressed as mean ± SD (n=3). Values within the same column with different letters are significantly different at $p < 0.05$ using Duncan's test.

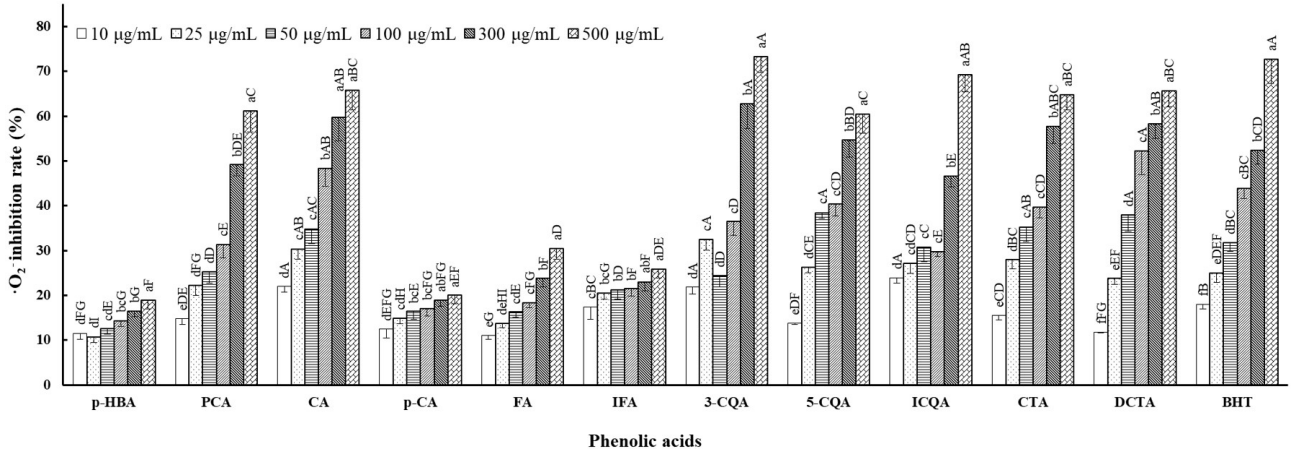


Figure 1. The inhibition rate of superoxide anion radicals of phenolic acids. Each value is expressed as mean ± standard deviation (n =3). Different lowercase mean significant differences for different concentrations at same phenolic acid, different capital letters mean significant differences for different phenolic acids at same concentration (p<0.05).

researchers, while its isomer IFA had rarely been reported before (Itagaki et al., 2009; Zhao & Moghadasian, 2008). In present study, FA and IFA were showed better scavenging activities of ·ABTS$^+$ than that of DPPH· with the same order as FA> IFA.

3.2 Superoxide anion and hydroxyl radical scavenging activity

Superoxide anion ($\cdot O_2^-$) and hydroxyl radical (·OH) were reactive oxygen species (ROS) known as important free radicals in living cells. Superoxide anion was considered as the precursor to active free radicals such as hydroxyl radical, hydrogen peroxide and singlet oxygen that may react with biomacromolecules and cause tissue injury. Figure 1 showed the scavenging activity on $\cdot O_2^-$ of phenolic acids and BHT at different concentrations. It was found that the scavenging activity increased with the increasing of the concentration of each phenolic acid ranged from 10 to 500 μg/mL. The $\cdot O_2^-$ scavenging activities of phenolic acids were close to or lower than BHT. The research by Li et al. (2011) showed PCA had lower scavenging activity at the concentration ranged from 50 to 300 μg/mL than BHT, which was in accordance with our findings. PCA, CA, DCTA, CTA and chlorogenic acid isomers including ICQA, 3-CQA and 5-CQA were showed better inhibition rates on $\cdot O_2^-$ than FA, IFA, *p*-CA and *p*-HBA, which could be explained by the theory that the number of hydroxyl groups in the aromatic ring of phenolic acids had close relationship with antioxidant activities (Adefegha et al., 2015).

As shown in Figure 2, inhibition activities of phenolic acids on ·OH were also exhibited in concentration-dependent manner. Except for 5-CQA, phenolic acids showed better inhibition activities than BHT at the concentration of 25 μg/mL. According to the Maurya's research, caffeic acid and ferulic acid started to behave as prooxidants when the concentration higher than 5 μg/mL, which could be explained by their iron reducing property (Maurya & Devasagayam, 2010). There was no prooxidant effect detected for caffeic acid and ferulic acid in this study.

3.3 FRAP activity

The increased absorbance showed the increased ferric reducing power of phenolic acids. As shown in Figure 3, ferric reducing power of phenolic acids raised with the increasing concentrations of phenolic acids from 1 to 25 μg/mL except *p*-HBA. *p*-HBA with mono hydroxylation structure was the only phenolic acid showed the lower reducing power than BHT at the concentration of 25 μg/mL. CA showed the highest value at the concentration of 25 μg/mL among phenolic acids, followed by PCA. The values of ICQA, 3-CQA and 5-CQA were very close to each other, which was in agreement with the former study by Xu et al. (2012) The values of FA and IFA were just a little lower than ICQA, 3-CQA and 5-CQA. DCTA showed significantly better activity than CTA at the concentrations ranged from 5 to 25 μg/mL, which could be related to the number of hydroxy groups in their structure.

3.4 β-carotene bleaching inhibition

The antioxidant activities of phenolic acids were also measured by the β-carotene bleaching method carried out in linoleic

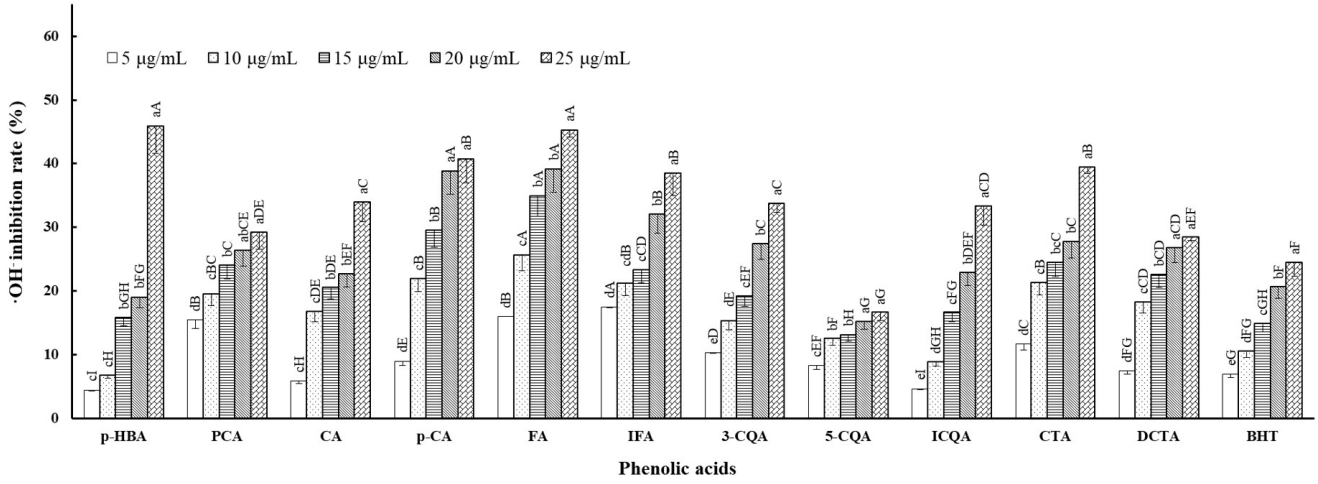


Figure 2. The inhibition rate of hydroxyl radicals of phenolic acids. Each value is expressed as mean ± standard deviation (n =3). Different lowercase mean significant differences for different concentrations at same phenolic acid, different capital letters mean significant differences for different phenolic acids at same concentration (p<0.05).

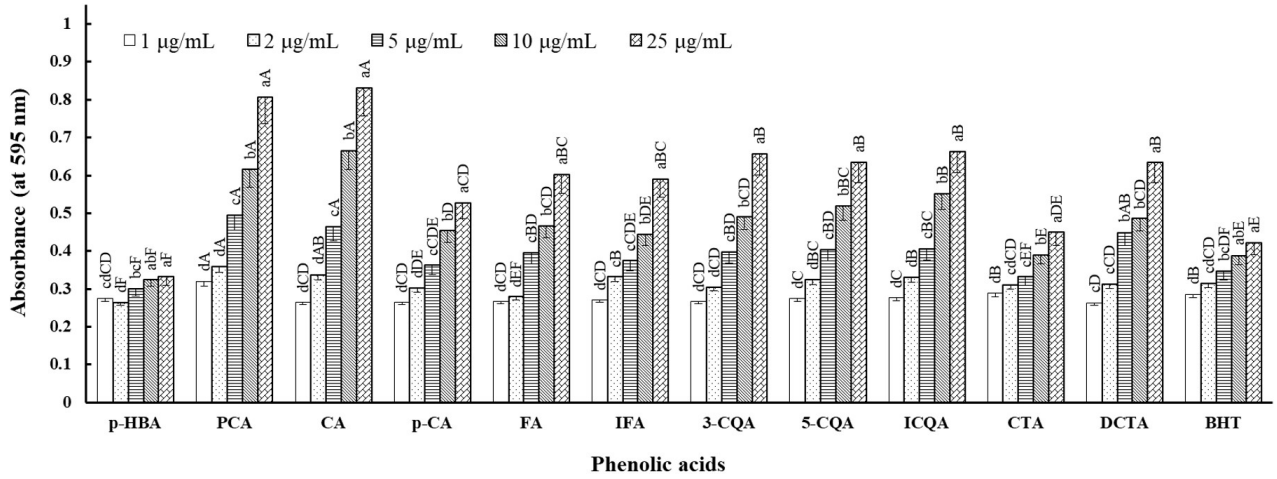


Figure 3. Ferric reducing antioxidant power of phenolic acids. Each value is expressed as mean ± standard deviation (n =3). Different lowercase mean significant differences for different concentrations at same phenolic acid, different capital letters mean significant differences for different phenolic acids at same concentration (p<0.05).

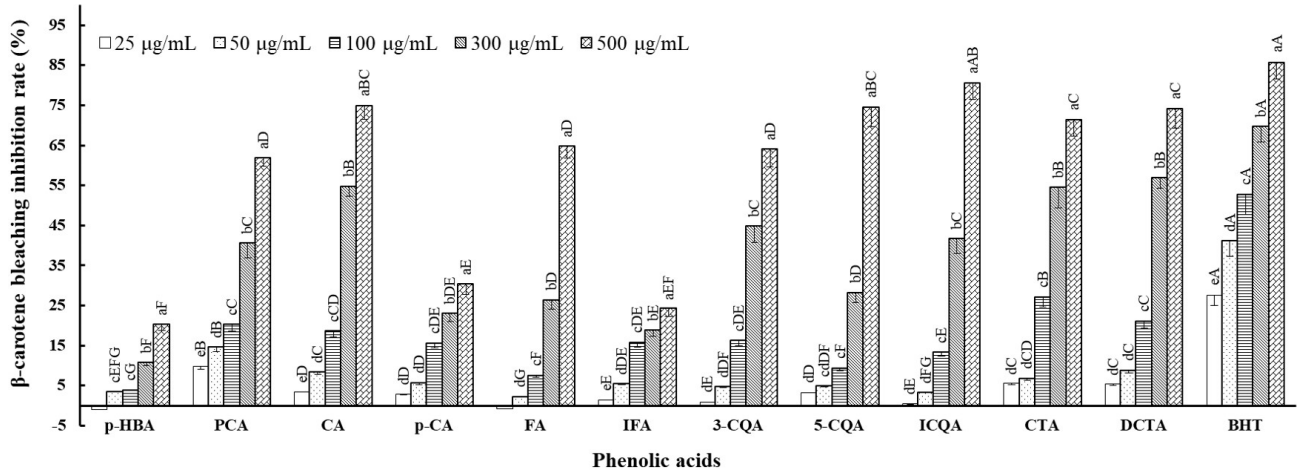


Figure 4. The inhibition rate of β-carotene bleaching of phenolic acids. Each value is expressed as mean ± standard deviation (n =3). Different lowercase mean significant differences for different concentrations at same phenolic acid, different capital letters mean significant differences for different phenolic acids at same concentration (p<0.05).

acid emulsion system. Phenolic acids can inhibit β-carotene bleaching by neutralizing hydroperoxides and other compounds derived from linoleic acid oxidation. As shown in Figure 4, the increasing inhibition rate of phenolic acids was observed with the increasing concentrations from 25 to 500 μg/mL. None of the phenolic acids measured in this study showed higher inhibition activity of β-carotene bleaching than BHT. The inhibition rate of chlorogenic acid isomers decreased in the order of ICQA (80.6%), 5-CQA (74.5%), and 3-CQA (64.1%), which may be related to the number of dihydroxylation structure in phenolic acids. This was in accordance with the former results by Xu et al. (2012). *p*-CA, *p*-HBA and IFA showed distinctly lower activities than other phenolic acids may be connected to the number of hydroxyls in phenolic acids. The pro-oxidant effect was observed only in FA and *p*-HBA at the concentration of 25 μg/mL.

4 Discussion

In preliminary experiment, the absorbance of reaction mixture was observed to change during the detection of antioxidant activities. The absorbance measured by spectrophotometer would take a long time when multiple samples determined. In the assays of our study, 96-well plate and multimode reader were employed to measure the absorbance of reaction mixtures at the same time of different phenolic acids and concentrations. The results get by this method could be more accurate and easier to compare with each other. β-carotene bleaching inhibition activity was evaluated by the spectrophotometric measurement of β-carotene concentration changes in β-carotene/peroxyl radicals (LOO·) systems with and without antioxidant. Linoleic acid auto-oxidation at 50 °C in water bath and induced oxidation by AAPH at 25 °C were compared in our study. The reaction rate of the induced oxidation by AAPH was more stable than that of linoleic acid auto-oxidation. So the AAPH solution was used as a radical initiator in β-carotene bleaching assay.

Phenolic acids with high antioxidant abilities in one method also showed good abilities in other methods, but not for *p*-HBA that have good antioxidant ability in ·OH scavenging assay rather than other methods. Structure of phenolic acids was regarded as the important factor for their antioxidant activity. In our study, CA and PCA with dihydroxy groups showed approximate activities in many assays. Their antioxidant activities were much better than that of *p*-CA and *p*-HBA which have one hydroxy group only. The results were in agreement with the statement of Sroka, who considered the antioxidant activities of phenolic acids were correlated positively with the number of hydroxyl groups bonded to the aromatic ring (Sroka & Cisowski, 2003). Further analysis showed CA had a little better activity than PCA, which may be attributed to the different substituents on the benzene ring, -CH=CHCOOH can enhance the antioxidant activities of phenolic acids, compared with -COOH (Chen et al., 2020). Similarly, *p*-CA showed higher activities than *p*-HBA in ·$ABTS^+$ scavenging, FRAP and β-carotene bleaching assay.

FA with one hydroxyl group in its structure had been proved to have good antioxidant activities in various assays. It may be due to the ortho methoxy group which enhanced the antioxidant activity. In the present study, FA had higher antioxidant activities than *p*-CA and *p*-HBA in most assays, which was in accordance with the former research (Kikuzaki et al., 2002). Lower antioxidant activities of IFA were found than FA in DPPH· and ·$ABTS^+$ scavenging and β-carotene bleaching assay. It may be related to the effect of hydroxy group and methoxy group in the opposite position.

Moreover, acylation was considered to have influence on the antioxidant activity of phenolic acids. Both of CTA and DCTA formed by tartaric acid contained the acyl structure. In our study, CA had stronger activities than 5-CQA, 3-CQA and CTA in DPPH·, ·$ABTS^+$ scavenging and FRAP assays, but weaker activity than 3-CQA in ·O_2^- inhibition assay. It was considered that acylation was related to antioxidant activities of phenolic acids but the relationship was uncertain. In the study of Xu et al., dicaffeoylquinic acid had better antioxidant activity than caffeoylquinic acid, mainly owing to an increase of hydroxyl groups (Xu et al., 2012). Our results obtained were consistent with the former research, which indicated that ICQA

had better activity than 3-CQA and 5-CQA, and DCTA than CTA in most cases.

Additionally, phenolic acids had also been reported as pro-oxidants in some papers. *p*-HBA, *p*-CA and vanillic acid were found to promote the lipid peroxidation (Simić et al., 2007). Both of CA and FA started behaving as prooxidants when reached the concentration limit in fenton reaction (Maurya & Devasagayam, 2010). However, pro-oxidant activity of phenolic acids was rarely observed in different assays except for FA and *p*-HBA at the concentration of 25 μg/mL in β-carotene bleaching assay in the present research. According to the former research, pro-oxidant activities of phenolic acids were usually found in the biomolecule methods involving lipid peroxidation, DNA damage or a transition metal existed (Maurya & Devasagayam, 2010; Simić et al., 2007). Thus, further studies will be needed to provide more conclusive evidence.

5 Conclusion

Phenolic acids commonly existed in vegetables were evaluated for their potential antioxidant activities by different typical assays. CA and PCA with simple dihydroxylation structures showed effective antioxidant activities in most assays, while *p*-HBA had low activities in these methods except in ·OH scavenging assay and *p*-CA except in ·$ABTS^+$ and ·OH scavenging assays. The antioxidant activity of CA was higher than PCA and FA higher than IFA in most assays. Antioxidant activities of CTA, DCTA and derivates of chlorogenic acid with complex dihydroxylation structures were relatively high among phenolic acids. Compared with BHT, phenolic acids showed stronger antioxidant activities in DPPH· and ·$ABTS^+$ scavenging assays, and weaker in $\cdot O_2^-$ scavenging and β-carotene bleaching assays. The pro-oxidant effect of phenolic acids was rarely observed in the present study. The results were conducive to further research on potential biological activities of vegetables, and provide the information for fortified food developing with phenolic acids added as natural antioxidants.

Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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